J. Physiol. (1969), **201**, *pp.* 305–320
With 7 text-figures
Printed in Great Britain

CHANGES IN CONTRACTILE PROPERTIES OF DISUSED SOLEUS MUSCLES

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(*Received* 12 *August* 1968)

SUMMARY

1. The hypothesis that the pattern of activity of muscle can determine its contractile properties was tested in the case of the rat soleus subjected to chronic disuse.

2. In order to characterize the pattern of motor activity over long periods of time, a method of chronic EMG recording with indwelling electrodes was developed. Some characteristics of the EMG recorded from unrestrained animals are described.

3. Immobilization of the knee and ankle joints reduced aggregate EMG activity in the soleus to 5–15 % of control and resulted in a shift from tonic to a more phasic pattern of firing.

4. After 4 weeks of immobilization, speeding of the soleus mechanical properties was indicated by: shortened contraction time; decreased tetanus/twitch ratio; increased maximum rate of development of tetanic tension; and decreased fusion during a 5/sec tetanus.

5. Because the contractile properties of the soleus can be altered by a period of disuse with no change in innervation, neuronal control of the contractile mechanism depends in part on impulse activity.

INTRODUCTION

Experiments with cross-innervated muscles have shown that the contractile properties of mammalian fast and slow twitch fibres are partly controlled by the motoneurone (Buller, Eccles & Eccles, 1960*a*, *b*; Buller & Lewis, 1965*b*). The firing patterns of the motoneurones innervating the two types of muscle are strikingly different and might provide the key to an understanding of the mechanism of this control. Alternatively, this influence could be due to a neural action unrelated to propagated impulses (Buller *et al.* 1960*b*).

Motoneurones which innervate slow muscles are tonically active, producing a greater aggregate number of impulses over long periods of time than the phasically active motoneurones which innervate fast muscles. In the experiments to be described, immobilization of the rat soleus, a slow twitch muscle, resulted in diminished and more phasic activity. If conducted activity were important, this period of altered neuronal discharge or 'disuse', even with no change in innervation, should result in a 'speeding' of contractile properties.

Disuse has previously been produced by tenotomy (Vrbova, 1963*b*), denervation (Lewis, 1962; Eccles, Eccles & Kozac, 1962), spinal cord section, and/or dorsal root section (Buller *et al.* 1960*a*, *b*; Eccles, 1941). However, these procedures involve either drastic changes in resting muscle tension, interruption of the reflex arc, or transection of supra-segmental inputs to the arc. Results may be due, in large measure, to modification of intercellular relations which are unrelated to nerve impulses, or to cell injury of either muscle (Engel, Brooke & Nelson, 1966; McMinn & Vrbova, 1964; Tower, 1937*b*) or nerve (Barron, 1933; Young, 1966). Furthermore, an attempt to document the actual impulse traffic has been made only in the case of tenotomy (Vrbova, 1963*a*) and there is no quantitative information about the long-term functioning of these model systems.

In an attempt to circumvent these objections, we have effected a relative disuse of rat soleus muscles by immobilizing the knee and ankle joints (Solandt, Partridge & Hunter, 1943), and have documented the degree of disuse by chronic EMG recording with indwelling electrodes. After a period of disuse, contractile properties were measured with isometric techniques.

METHODS

Animals. Male, Osborne–Mendel rats were used in all experiments. Except where otherwise stated, animals included in the study of contractile properties weighed 150–200 g at the time of joint fixation and 275–350 g when sacrificed.

EMG. Chronic recordings were obtained from the soleus, extensor hallucis longus, and gracilis anterior. Recording was bipolar, with 34–36 gauge Teflon coated stainless-steel electrodes. The wires were bent at right angles near the tips and embedded in the muscle with the interelectrode path parallel to the long axis of the fibres. The recording surfaces were either the cut ends (125–160 μ diameter), or, in those cases in which the leads passed through the muscle, small cuts in the insulation. When recording was from the cuts, the tips were covered with silastic or dental cement. The interelectrode distance was kept constant by collaring the leads with a thin slip of Perspex or cellophane just before entry and on exit from the muscle. The wires were tied to adjacent fascia or tendon, loosely coiled, and then led subcutaneously to a connector over the lumbar area. A coiled, bare wire embedded subcutaneously near the back plug served as a ground lead. The plug was either anchored to two fused lumbar vertebrae with dental cement or to a stainless-steel metal plate sewn to the lumbar fascia.

The animal was connected to the recording equipment for 30 min each day by a long flexible cable which allowed relatively uninhibited movement about his cage. After amplification by an R-C coupled system with a bandpass of 800–10,000 c/s ± 3 db, all spikes above a pre-set level were counted electronically and registered with a suitable scaling factor on mechanical counters and on a pen writer. The low frequency cutoff minimized base line fluctuations which would have produced spurious counts. Samples of activity were stored on magnetic tape. We attempted to modify the diurnal activity cycle of those rats included in the EMG study with the intention of making daily counts more reproducible. They were kept under constant illumination and fed at a fixed time of day immediately after the recording period. Shirley (1928) reported that rats adapted to a similar regimen became most active around the hour before feeding. This general pattern was evident in one animal whose EMG was continuously monitored over several 24 hr periods by radiotelemetry.

Fixation. Ankle and knee joints were immobilized in approximately mid-position by driving 22- or 25-gauge needles through the calcaneous into the distal tibia and through the distal femur into the proximal tibia respectively (Solandt *et al.* 1943). Rats were anaesthetized with either halothane inhalation, where rapid recovery was desirable, or intraperitoneal chloral hydrate, 40–100 mg. Although the rats subsequently kept the limb elevated, they showed no obvious sign of pain and would occasionally bear weight on the immobilized leg. Their gain in body weight over the period of immobilization was only slightly less than the control group (100 versus 130 g).

Contractile properties. Mechanical properties were studied *in vitro* at 23–26° C with isometric techniques 2 and 4 weeks after limb immobilization. A Grass FT 10 strain gauge (compliance 5 mm/kg) was used in early experiments and a Statham gauge (compliance 0·5 mm/kg) in later studies. The amplified output was led to a pen writer before and after differentiation by an operational amplifier circuit. The muscles were stimulated through the bath by two large encircling platinum rings with a current strength greater than that required for maximum twitch response. The pulse duration was 0·6 msec. In no instance did two shocks delivered with progressively shorter delay produce a twitch tension which was less than that obtained with one shock alone, as expected if the muscle fired repetitively to a single shock (Buller & Lewis, 1963). Curare had little (*ca.* 10 % depression) or no effect on twitch tension.

The muscles were bathed in a solution composed of 125 mM-NaCl, 5 mM-KCl, 1 mM-$MgCl_2$, 1 mM-NaH_2PO_4, 2 mM-$CaCl_2$, 24 mM-$NaHCO_3$ and 11 mM glucose. The solution was bubbled with 95 % O_2–5 % CO_2. There was a linear relation between maximum tetanic tension of control muscles (mean = 120 g) and muscle wet weight over a range of muscle weights extending from 130 to 400 mg. In addition, tetanic tensions were comparable to a series (mean = 128 g) recorded by J. B. Wells (personal communication) from rat soleus muscle of rats indirectly stimulated *in vivo*. Therefore, it was assumed that oxygenation and stimulus strength were adequate.

During each experiment the muscle was maintained at that resting tension at which maximum twitch tension was obtained; a series of prolonged tetani, each tetanus at a progessively higher frequency, was applied until the maximum tetanic tension (P_0) was attained; and finally, a series of brief tetani (five shocks) at increasing frequencies was applied until the maximum rate of tension development was attained. The following were measured: (1) contraction time (the time at which the differentiated tension record returned to zero), (2) max. tetanus-twitch ratio (the ratio of P_0 to twitch tension), (3) 5/sec tetanus-twitch ratio (the ratio of the maximum tension attained during 5/sec stimulation to twitch tension), and (4) $\%P_0$/msec (the maximum rate of tetanic tension development normalized to maximum tetanic tension).

The duration of active-state plateau was estimated by the method of Macpherson & Wilkie (1954). The muscle was stimulated by five shocks separated by the interval at

which the peak rate of tension development was maximum. The differentiated tension record obtained under these conditions was summed by computer 20–30 times, and an equal number of differentiated records, obtained with one shock stimulation, was subtracted. In the resulting trace, the first deflexion marked the earliest separation of one and five shock derivatives. The separation time is a measure of the duration of active-state plateau (Macpherson & Wilkie, 1954).

Controls. A soleus muscle contralateral to an immobilized limb showed no change in EMG activity (Fig. 3). Moreover, contralateral muscles did not differ from muscles from unaltered animals in either dry weight or in any contractile property studied. Therefore, they were included in the control group in all significance tests.

RESULTS

Electromyographic findings in normal and disused muscles. Potentials large enough to be counted ($> 20\,\mu V$) originated from motor units of the muscle in which the leads were implanted; there was no cross-talk from adjacent muscles. Immediately after electrode placement, section of the motor nerve usually eliminated all discrete spikes. Activity from adjacent muscles merely increased the base line noise. On occasion, small discrete spikes remained after the nerve was cut, but they were well below the counting level (see Methods). In the animals monitored for long periods, action potentials always correlated with the expected action of the muscle.

We distinguished spikes from different motor units on the basis of size, configuration and firing pattern. The same unit could usually be identified for several days, and on occasion, for several weeks (Fig. 1*A* and *B*). The configuration of some units changed gradually over several days (Fig. 1*C*). Small changes in the relative synchrony of components of the unit, in addition to tissue reaction near the electrodes, probably explain this evolution.

Most of the units from the soleus were readily distinguished from those of fast twitch muscles by three criteria: (1) *aggregate*: a greater number of impulses over long periods of time; (2) *pattern*: more continuous firing; and (3) *frequency*: longer interspike intervals during periods of activation. Soleus units fired tonically at 5–20/sec, whereas extensor hallucis longus and gracilis anterior units fired intermittently at 10–70/sec with occasional bursts at frequencies as high as 125/sec. However, the soleus exhibited occasional units which were only phasically active during periods of intense activity, and some units from 'phasic' muscles fired regularly for prolonged periods (Fig. 1*D*).

Following immobilization (see Methods) of the knee and ankle, the aggregate number of spikes in the soleus was depressed to 5–15% of control (Figs. 2 and 3). Counts were reduced at the earliest time measured (4 hr post-operative) and remained depressed for 4 weeks. The joints in

one animal whose counts (not shown in Fig. 3) returned toward normal were not adequately fixed. The pattern of activity was also strikingly altered (Fig. 2), changing towards that of a phasic muscle, i.e. there were long silent periods interrupted by bursts of spikes (Fig. 2*c*).

Figure 4 illustrates the fact that changes in total spike count and pattern of firing of the entire population reflected similar changes in individual motor units. The stability of unit size and configuration insured that alterations in spike counts following immobilization were not due to changes in recording conditions.

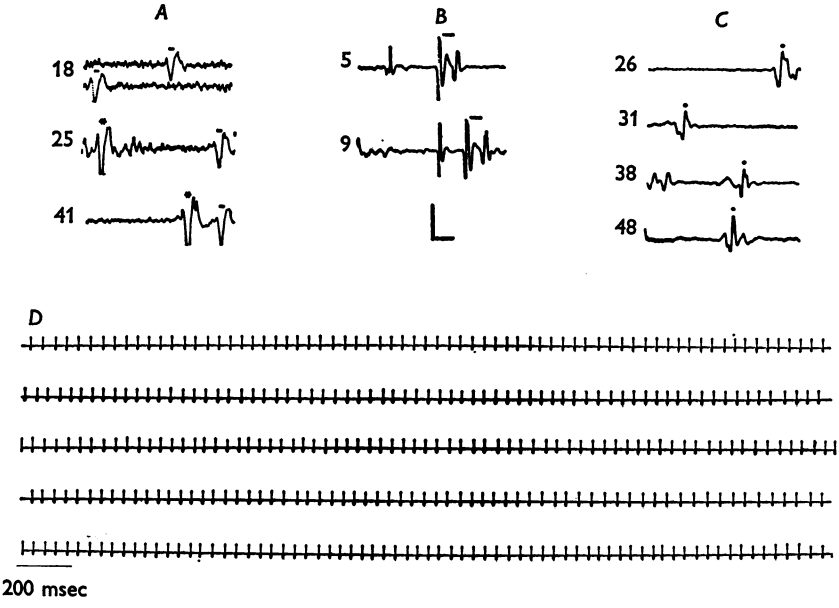

Fig. 1. Motor unit potentials from unrestrained rats. *A*, extensor hallucis longus; *B* and *C*, gracilis anterior. Numbers to the left of each trace refer to days after electrode implantation. Different symbols over different units. Note the gradual change in shape of the unit in *C*. Vertical bar: *A* 80 μV, *B* and *C* 400 μV. Horizontal bar: 8 msec. *D*, regularly firing unit from the gracilis anterior. Successive lines from a continuous record.

Despite these drastic changes in aggregate and pattern, after 2 weeks of disuse the frequencies of individual units during periods of activation were unchanged. The mean control interspike interval was $97{\cdot}6 \pm 10{\cdot}0$ (S.E. of mean) msec ($N = 17$), whereas for disused muscles it was $89{\cdot}0 \pm 4{\cdot}9$ msec ($N = 19$). Four units followed for 4 weeks fired at twice their control rate but still within the range of units from unaltered solei and far below frequencies characteristic of units from fast muscles.

Although not investigated in detail, it appears that trains of impulses from motor units in spontaneously active animals cannot be considered stationary point processes. Therefore, estimates of mean interspike intervals from small samples of data are, at best, approximate. A unit was considered 'on' and 25–100 consecutive intervals were measured when the coefficient of variation of the intervals was less than 0·3, i.e. when it fired regularly.

Attempts to alter activity in the phasic extensor hallucis longus and gracilis anterior muscles by various sling and cast arrangements or by internal fixation led to inconsistent results and were abandoned.

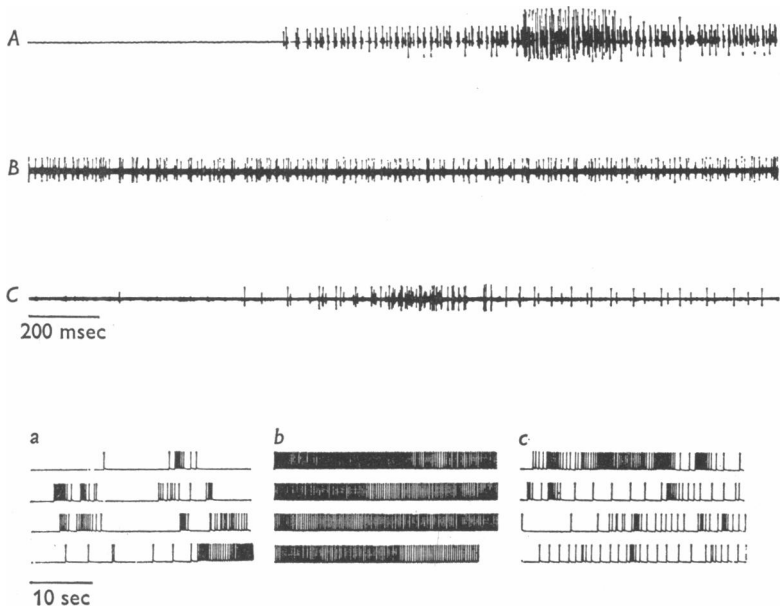

Fig. 2. Representative EMGs from (*A*) gracilis anterior, (*B*) normal soleus, (*C*) soleus after immobilization of knee and ankle joints. Pen writer records (*a*, *b* and *c*) on a slower time scale, correspond to *A*, *B* and *C* respectively. Each pen deflexion represents twenty motor unit spikes. Note the orderly recruitment from small to large potentials in *A* (cf. Olson, Carpenter & Henneman, 1966). Following immobilization (*c*), the pattern of activity more closely resembles that of the phasic muscle (*a*) than that of the normal tonic soleus (*b*).

Contractile properties. Contractile properties were studied (see Methods) in six disused and nine control soleus muscles 4 weeks after pinning. The wet and dry weights of the disused muscles were approximately one-half control, the maximum tetanic tension less than one-third. Wet weights were linearly related to dry weights. Because the maximum tetanic tension per mg dry weight was less for atrophic than control muscles, P_0 (maximum tetanic tension) instead of P_0/cross-sectional area or P_0/mg was taken as the best measure of the output of contractile sites acting in

parallel. The rates of tension development were accordingly normalized to $\%P_0$/msec.

The disused muscles exhibited contractile properties intermediate between normal 'slow' solei and normal 'fast' muscles (Figs. 5 and 6; Table 1). The maximum rate of tetanic tension development (max. $\%P_0$/msec) was increased.

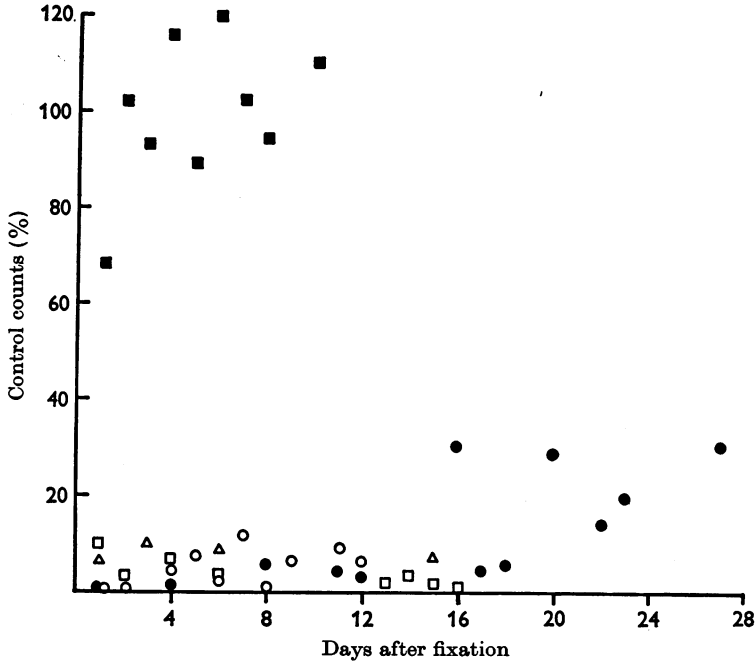

Fig. 3. Depression of soleus motor unit activity following immobilization of knee and ankle joints. Counts are expressed as percent of mean of daily counts before immobilization. Filled squares represent counts from a muscle contralateral to an immobilized leg. Each symbol stands for a different animal. ■, contralateral soleus; □ △ ● ○, soleus on pinned side.

If, as a first approximation, the fully active tetanized muscle is thought of as a contractile component in series with a non-linear, undamped elasticity (Hill, 1949), then the rate of tension development under isometric conditions is

$$\frac{dP'}{dt} = \frac{dP'}{dx}\frac{dx}{dt},$$

where P' is $\%P_0$, dP'/dx is the reciprocal of the series elastic compliance and dx/dt is the rate at which the compliance is stretched, presumably equal to the velocity of shortening of the contractile component (but see Jewell & Wilkie, 1958). J. B. Wells (personal communication) found,

by quick release experiments, that the compliance ($1/dP/dx$) of rat soleus muscles immobilized for 4–6 weeks by a different method of joint fixation was unchanged. Therefore, the increased max. % P_0/msec in disused muscles must reflect an increase in dx/dt.

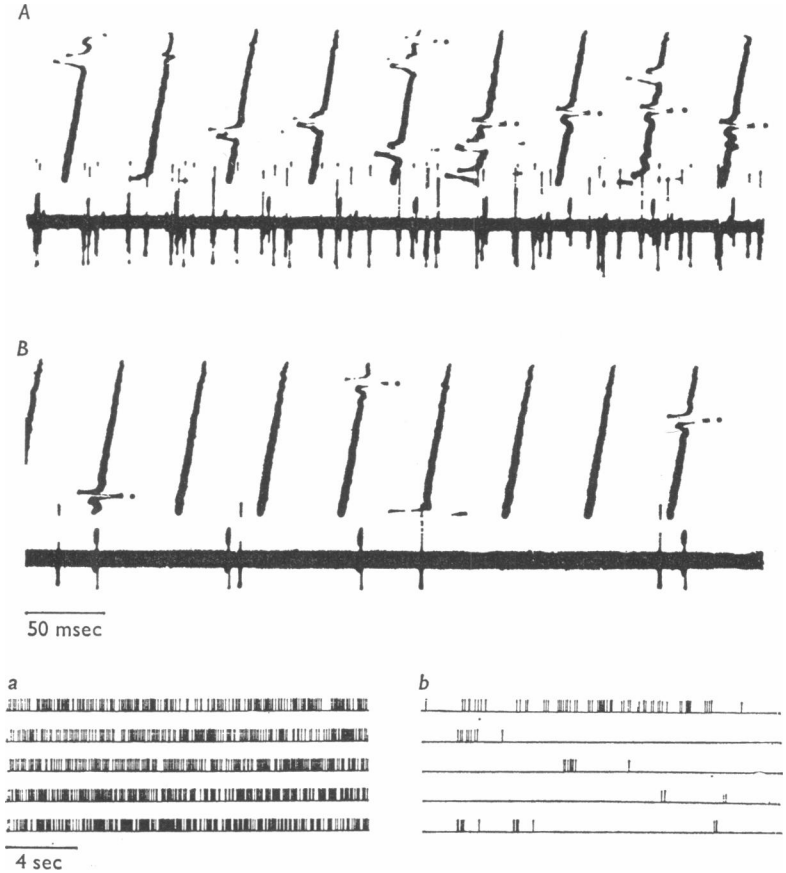

Fig. 4. Representative example of the effect of immobilization on the same motor unit followed over several days. Spontaneous activity. *A* control, before joint immobilization; *B* two days after immobilization. Vertical sweeps are simultaneous samples of the EMG displayed on a faster time base. The same unit is darkened on the horizontal traces and indicated with a dot on the vertical sweeps. The spikes of the unit darkened in *A* and *B* were selected on the basis of amplitude by a 'window' device and are displayed in pen writer records *a* and *b*, respectively. Each pulse in the pen writer records represents twenty spikes.

The increase was observed not only at the maximum, but, as shown in Fig. 7, at all tensions throughout the course of tetanic tension development. This result implies a shift in the entire force-velocity locus.

The observed combination of (1) shortened contraction time and (2)

decreased tetanus/twitch ratio is, in the context of the classic model of the isometric twitch (Hill, 1949, Fig. 7; Ritchie, 1954), consistent with an increased rate of shortening of the contractile component. An increased tetanus/twitch ratio would have been expected if the faster twitch time resulted solely from an abbreviated active state time course.

The duration of the active-state plateau was estimated in three animals (see Methods). The earliest separation of the differentiated tetanic and

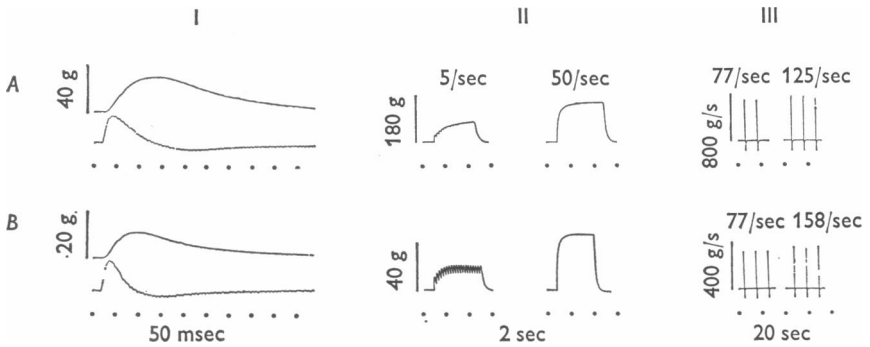

Fig. 5. Mechanical properties of control (*A*) and disused (*B*) muscles; isometric recording, *in vitro*, at 24–26° C. Column I: upper traces of each pair are isometric twitches; lower traces differentiated records. Column II: tetani at indicated frequencies. The 50/sec tetanus represents the maximum tetanic tension in both these cases. Column III: derivatives of tension rise during tetani at indicated frequencies. The maximum rates of tension development occurred at 125/sec (*A*) and 158/sec (*B*). Rates expressed here as g/sec.

twitch records occurred at similar times in disused muscles (9·9 and 8·9 msec) and controls (9·7, 7·5 and 7·0 msec). However, a faster rate of active-state decay has not been ruled out. The muscles relaxed faster as indicated by a decreased tetanus/twitch ratio when stimulated at 5/sec. In so far as relaxation is a function of active state, this argues for a faster decay.

Muscles immobilized for only *two* weeks showed atrophy (58 % control dry weight), decreased maximum tetanic tension (61 % control) and decreased maximum tetanus/twitch ratios. The contraction times and 5/sec tetanus/twitch ratios were unchanged at this time (Table 1). The rate of tetanic tension development was not measured. The faster control twitch times observed at this time compared with the 4-week control probably reflect the younger age of the animals (Close, 1964 and see below).

In all experiments described above, the limbs were immobilized in 10-week-old rats and tested in 12- or 14-week-old rats. Close (1964) reported, and our results, obtained at room temperature, confirm that normal solei show a lengthening of contraction time during this period

of development. The question arises whether the results of immobilization are due to an actual speeding of muscle properties or to an arrest of normal development. In order to settle this point, we measured the contractile properties of muscles from 18-week-old rats and established that no further developmental changes occur beyond 14 weeks of age. Three solei from hind limbs immobilized at 14 weeks showed significant

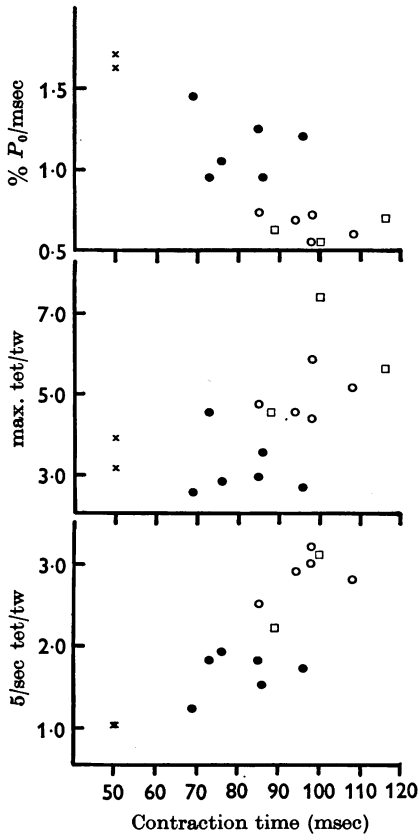

Fig. 6. Effects of limb immobilization on contractile properties. From top downwards, ordinates are maximal rate of tension development (% P_0/msec), maximum tetanus-twitch ratio (max. tet/tw), and tetanus-twitch ratio at tetanizing frequency of 5/sec (5/sec tet/tw). Each point is from one muscle. ●, represents soleus pinned, ○ soleus contralateral, □ soleus unaltered and × plantaris and peroneus longus.

speeding by all the criteria described above when tested at 18 weeks. Therefore, the effects of immobilization cannot be attributed to developmental arrest.

TABLE 1. Contractile properties of normal and chronically immobilized soleus muscles at 25° C. Numbers in parentheses refer to numbers of muscles in each group. Results are expressed as mean ± S.E. Probabilities are from the t distribution; values of t are based on a pooled estimate of the variance

	Dry wt. (mg)	Twitch tension (g)	Tetanic tension (g)	Twitch time (msec)	Max. tet/tw	5/sec tet/tw	$\%P_0$/msec
Control	33·2 ± 1·1 (7)	23·7 ± 2·1 (8)	120·6 ± 7·5 (8)	98·5 ± 3·5 (8)	5·3 ± 0·4 (8)	2·9 ± 0·2 (8)	0·65 ± 0·09 (8)
Immobilized 4 weeks	17·5 ± 1·5 (5)	16·8 ± 2·3 (6)	50·4 ± 5·4 (6)	80·8 ± 5·4 (6)	3·1 ± 0·3 (6)	1·7 ± 0·1 (6)	1·15 ± 0·22 (6)
Probability	< 0·001	< 0·025	< 0·001	< 0·010	< 0·005	< 0·001	< 0·001
Control	25·5 ± 0·6 (12)	18·8 ± 0·8 (13)	96·0 ± 8·0 (10)	79·6 ± 2·9 (13)	5·6 ± 0·2 (13)	1·7 ± 0·1 (13)	—
Immobilized 2 weeks	15·0 ± 1·3 (5)	16·0 ± 1·5 (5)	58·7 ± 9·6 (3)	76·3 ± 8·7 (4)	3·9 ± 0·4 (5)	1·9 ± 0·2 (5)	—
Probability	< 0·001	> 0·1	< 0·05	> 0·5	< 0·001	> 0·2	

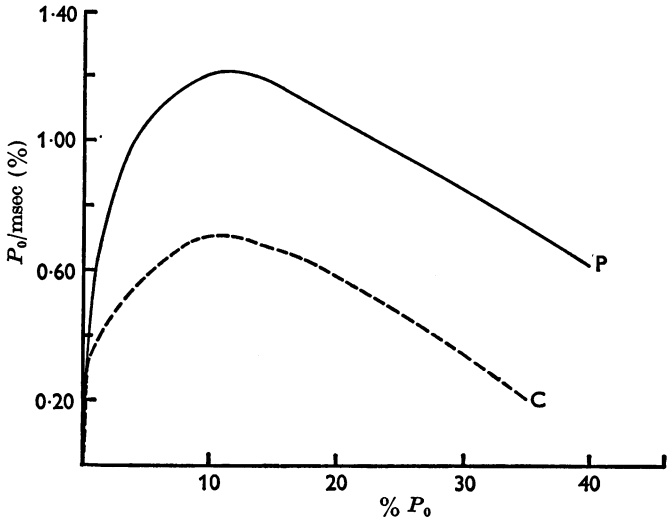

Fig. 7. 'Phase plot' of short tetani from immobilized (P) and control (C) soleus muscles. The rate of tension development during a tetanus is displayed as a function of tension. In both P and C, the stimulus frequency was chosen so that the peak rate of tension development was equal to 'max. % P_0/msec'.

DISCUSSION

The contractile properties of soleus muscles immobilized for 4 weeks change toward those characteristic of fast twitch muscles. Two lines of evidence point to an increased speed of shortening as the mechanism underlying this change, (1) an increased rate of tetanic tension development and (2) the combination of shortened contraction time and decreased tetanus/twitch ratio. However, the disproportionate decrease in tetanus/twitch ratio compared to the increase in $\%P_0$/msec, and the diminished fusion during a 5/sec tetanus suggest a somewhat more rapid decay of the active state. The rate of onset of the active state, perhaps an important determinant of contraction speed, was not studied.

Objections have been raised to the assumptions underlying the traditional interpretation of the isometric myogram (Jewell & Wilkie, 1958; Podolsky, 1960; Pringle, 1960). Therefore, attempts to characterize rigidly the change in disused muscles as one of either active state or velocity of shortening seem of limited heuristic value. Indeed, the two parameters may not be independent (Close, 1964, 1965, 1967). Nevertheless, the contraction time, maximum rate of tetanic tension development, and maximum and 5/sec tetanus/twitch ratios are indicators that distinguish fast from slow twitch muscles. As such, they were useful in the present study in demonstrating a 'speeding' of the mechanical properties of slow

muscles subjected to disuse. Significant alterations in these measures require explanation even though the values found in disused slow muscles were by no means identical to those of normal fast muscles.

It is possible that chronic change in predominant resting tension can account for the altered mechanical properties of disused muscle. However, in the cat, tenotomized muscles, which undergo more drastic changes in resting tension, do not show a change in contractile properties (Nelson, 1969). Furthermore, the muscles were immobilized in mid-position to minimize this factor.

Speeding of the immobilized soleus might have resulted from preferential atrophy of the slower motor units in a mixed population containing some faster units (Close, 1967). However, histological examination of the muscles showed a uniform atrophy of all fibres. Also, no evidence of a composite or broad-humped twitch was seen, whereas this would have been expected if two fibre types with widely divergent contraction times each contributed substantially to total twitch tension (Biscoe & Taylor, 1967; Robbins, Karpati & Engel, 1969).

If the contractile component of immobilized muscles atrophied out of proportion to the atrophy of the series elastic component, apparent speeding might occur because of the relatively less compliant series elastic (Buller & Lewis, 1965*a*). However, muscle atrophy does not necessarily lead to speeding (e.g. twitch times at 2 weeks, Table 1; Nelson, 1969), and direct measurement of the series elastic compliance in atrophic soleus muscles revealed no change from normal (J. B. Wells, personal communication).

Histochemical findings also point to a genuine change in muscle fibre type, rather than a specific atrophy. In the rat, Type I fibres, as defined by the myofibrillar ATPase reaction, predominate in the normal soleus; Type II fibres predominate in normal fast muscles. Four weeks after immobilization, soleus muscles showed a reversal in the ratio of Type I and Type II fibres (M. Brooke, personal communication). Moreover, this reversal, with its possible physiological implication (Robbins *et al.* 1969) was not accompanied by the severe degenerative changes noted after tenotomy (McMinn & Vrbova, 1964; Engel *et al.* 1966).

With respect to contractile properties, denervation and disuse have opposite effects. Chronic disuse, as reported here, results in speeding whereas denervation results in slowing of contractile properties (Eccles *et al.* 1962; Lewis, 1962; Slater, 1966). Clearly, the effect of denervation cannot be ascribed to disuse.

It is difficult to specify which features of motor unit activity are important in the determination of contraction speed. The most striking changes in the EMG study were the profound decrease in total counts and the shift in firing pattern from the continuous barrage of the normal

soleus to the bursts characteristic of a fast muscle. These are the most likely causes of the altered mechanical properties. Experiments involving tenotomy or spinal cord isolation provide data consistent with this interpretation. The rabbit soleus tenotomized for at least 4 weeks shows significant speeding of contractile properties (Buller & Lewis, 1965*a*). In this case, Vrbova (1963*a*) reported a decrease in aggregate motor unit activity. The same procedure in the cat does not alter EMG counts and does not result in a change in contraction time (Nelson, 1969). Spinal cord isolation which results in both diminished number of impulses and occasional bursts of activity (Johns & Thesleff, 1961; Tower, 1937*a*) also produces speeding of slow muscle (Buller *et al.* 1960*b*).

In like manner, the effects of cross-innervation on contractile properties may depend on the activity pattern imposed on the muscle by the cross-innervating motoneurone. And the existence of motor units with different firing patterns (e.g. Fig. 1*D*) may serve to maintain the bimodal distribution of unit contractile properties in normal muscles (Wuerker, McPhedran & Henneman, 1965; Burke, 1967; Close, 1967).

In other experiments (Vrbova, 1966; Vrbova & Salmons, 1967), frequency of firing rather than pattern or aggregate appears to be the determining factor. But in the light of the present study, a change in frequency cannot be considered a necessary condition for a change in contractile properties.

How might activity affect contractile properties? An attractive hypothesis is that the nerve exerts a 'trophic' influence on muscle metabolism and physiology and that this influence is related to conducted electrical activity. Indeed, since activity is known to affect neuronal biochemistry and structure (Hyden, 1960; Tobias & Nelson, 1959) and axonal transport of material (Kerkut, Shapira & Walker, 1967), it would be surprising if a trophic influence were independent of activity. Furthermore, the fact that some trophic effects can be disassociated from nerve activity (Gutmann, 1964) does not imply that these or other trophic effects are normally independent of activity patterns.

Another attractive hypothesis is that the nerve influences the muscle indirectly; it imposes a specific pattern of mechanical stress on the muscle. Different patterns of stress, in turn, might regulate the contractile apparatus by calling into action different biochemical control mechanisms either directly or as a result of altered blood flow. It is at present impossible to distinguish the effects of activity dependent neurotrophic influences from those of active muscle exercise since the two are *pari passu*. Experiments employing direct stimulation of large mammalian muscle would not dissociate these factors because the large currents required probably stimulate nerve endings and possibly cause additional reflex effects.

Whatever the mechanisms involved, the present experiments demonstrate that the speed of the contractile apparatus is subject to considerable change with no change in innervation. The role of the nerve in determining contractile properties is not dependent on some factor unique to a particular class of motoneurones. If a neurotrophic influence is at work, it is modifiable and partially dependent on neuronal activity.

The authors wish to thank Drs Karl Frank, P. G. Nelson and J. B. Wells for many valuable criticisms.

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